

Data Evaluation Report on the Acute Toxicity of STABILAN (a.i. Chlormequat chloride) to *Scenedesmus subspicatus*

PMRA Submission Number {.....}

EPA MRID Number 467152-22

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D325185
	OECD Data Point	{.....}
	EPA MRID	467152-22
	EPA Guideline	OPPTS 850.5400

Test material:	STABILAN	Purity: 465 g/L
Common name:	Chlormequat chloride	
Chemical name:	IUPAC: 2-chloro-N,N,N-trimethylethan-ammoniumchloride salt	
	CAS name: Not reported	
	CAS No.: Not reported	
	Synonyms: Not reported	

Primary Reviewer: Brian D. Kiernan
OPP/EFED/ERBIV

Date: 04/24/06

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	018101	

Date Evaluation Completed: {dd-mm-yyyy}

CITATION: Wuthrich, V. 1990. Acute toxicity of Stablian to *Scenedesmus subspicatus* (OECD-Algae growth inhibition test). Unpublished study performed by RCC Umweltchemie AG, Itigen/BL, Switzerland. Study No. 1990/7001881. Study sponsored by BASF Corporation, Agricultural Products, Research Triangle Park, NC. The final report issued August 31, 1990.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic nonvascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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*Scenedesmus subspicatus***

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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Scenedesmus subspicatus* were exposed to Chlormequat chloride (as STABILAN) at nominal concentrations of 0 (negative control), 62.5, 125, 250, 500, and 1000 mg product/L. Nominal concentrations of 62.5, 250, and 1000 mg/L were analytically verified at 0 and 96 hours; mean measured concentrations of the product STABILAN were 47.89, 232.6, and 898.9 mg/L.

Neither cell density nor area under the growth curve (biomass) exhibited inhibition in excess of 50%. The EC_{50} values were >898.9 mg/L (based on measured concentrations). By 96-hours, cell density percent inhibition was -2, 8, 4, 13, and 15% at the nominal 62.5, 125, 250, 500, and 1000 mg/L treatment levels, respectively, compared to the control. The NOAEC for cell density was 898.9 mg/L. The area under the growth curve (biomass) percent inhibition was -6.5, 6.8, -0.9, 12.0, and 16.2% at the nominal 62.5, 125, 250, 500, and 1000 mg/L treatment levels, respectively, compared to the control. The NOAEC for biomass was 232.6 mg/L.

This toxicity study is scientifically sound and satisfies the guideline requirement for aquatic nonvascular plant toxicity study with the freshwater green algae species, *Scenedesmus subspicatus*. This study is classified as ACCEPTABLE.

Results Synopsis

Test Organism: *Scenedesmus subspicatus*

Test Type (Flow-through, Static, Static Renewal): Static

Cell density:

EC_{05} :	140 mg/L	95% C.I.: 10-1800 mg/L
EC_{50} :	>898.9 mg/L	95% C.I.: Not calculable
NOAEC:	898.9 mg/L	
Probit Slope:	0.834 ± 0.477	

Area under the growth curve (biomass):

EC_{05} :	210 mg/L	95% C.I.: 33-1300 mg/L
EC_{50} :	>898.9 mg/L	95% C.I.: Not calculable
NOAEC:	232.6 mg/L	
Probit Slope:	1.18 ± 0.641	

Endpoint(s) Affected: Cell density and biomass

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test protocol was based on the OECD Guideline 201, "Algae, Growth Inhibition Test". The following deviations from U.S. Environmental Protection Agency Series 850-Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.5400, *Algal Toxicity, Tiers I and II* were noted:

1. The dilution water characteristics of source/type, TOC, particulate matter, metal, pesticide, and chlorine content were not reported.
2. The type of culture medium was not reported.
3. The pre-test health of the algae cultures was not reported.
4. Test concentrations were not analytically determined at test initiation and termination for all treated levels.

These deviations did not affect the validity of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The test was conducted according to the US EPA Good Laboratory Practice Standards 40 CFR, Part 160.

A. MATERIALS:

1. Test material Chlormequat chloride

Description: White liquid

Lot No./Batch No. : 2859969

Purity: 465 g/L

Stability of compound under test conditions: The stability of the test substance in the dilution water during the course of the study was demonstrated by analytical determinations at 0 and 96 hours for the 62.5, 250, and 1000 mg/L treatment groups. The mean recoveries were 79.1-92.1% of nominal at 0 hour and 74.1-94.0% of nominal at 96 hours.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Stored at -20°C, protected from light.

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Physicochemical properties of CHLORMEQUAT CHLORIDE

Parameter	Values	Comments
Water solubility at 20EC	10 ⁶ mg/L	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Name: *Scenedesmus subspicatus*

EPA requires a nonvascular species: For tier I testing, only one species, *S. capricornutum*, to be tested; for tier II testing, *S. costatum*, *A. flos-aquae*, *S. capricornutum*, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: *S. capricornutum*, *S. subspicatus*, and *C. vulgaris*. If other species are used, the strain should be reported

Strain: Not reported

Source: Culture maintained at study laboratory (original supplier was Umweltbundesamt, Berlin, Germany).

Age of inoculum: 3 days old

Method of cultivation: Nutrient medium (not specified)

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: The definitive test concentrations were based on range-finding studies. The results of the non-GLP range-finding studies were not reported.

b. Definitive Study

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Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period:	Continuous culture	<p><i>EPA recommends two-week acclimation period.</i></p> <p><i>OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.</i></p>
Culturing media and conditions: (same as test or not)	Nutrient medium (not specified); same as test.	
Health: (any mortality observed)	Not reported	
<u>Test system</u> Static/static renewal	Static	<p><i>EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).</i></p>
Renewal rate for static renewal	N/A	
Incubation facility	Environmental chamber	
Duration of the test	96-hours	<p><i>EPA requires: 96-120 hours</i></p> <p><i>OECD: 72 hours</i></p>
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	Erlenmeyer flasks 50 mL 30 mL	<p><i>OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.</i></p>

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<u>Details of growth medium name</u> pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	Nutrient medium 7.6 6.5-7.0 Not reported Not reported N/A	<p>-----</p> <p><i>OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.</i></p> <p><i>EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91 and D 3978-80 (reapproved 1987).</i></p>
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
<u>Dilution water</u> source/type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Not reported 7.6 N/A pH-adjusted Not reported Not reported Not reported Not reported Not reported	<p>The dilution water characteristics of source/type, TOC, particulate matter, metal, pesticide, and chlorine content were not reported.</p> <p>-----</p> <p><i>EPA pH: <u>Skeletonema costatum</u> = ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.</i></p> <p><i>OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.</i></p>
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solutions	
Aeration or agitation	Continuously agitated at 120 rpm.	
Initial cells density	Approximately 10,000 cells/mL.	

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		<p><i>EPA requires an initial number of 3,000 - 10,000 cells/mL. For <i>Anabaena flos-aquae</i>, cell counts on day 2 are not required.</i></p> <p><i>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <i>S. capricornutum</i> and <i>S. subspicatus</i>. When other species are used the biomass should be comparable.</i></p>
<p><u>Number of replicates</u></p> <p>Control: 3</p> <p>Solvent control: N/A</p> <p>Treatments: 3</p>		<p><i>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <i>Navicula</i> sp. tests should be conducted with four replicate.</i></p> <p><i>OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.</i></p>
<p><u>Test concentrations</u></p> <p>Nominal:</p> <p>Measured:</p>	<p>0 (negative control), 62.5, 125, 250, 500, and 1000 mg/L.</p> <p><26.25 (<LOQ, negative control), 47.9, 233, and 899 mg/L</p>	<p>An additional 1000 mg/L treatment group without algae was maintained under test conditions.</p> <p>The mean measured concentrations are based on the measured concentrations for the low (62.5 mg/L), medium (250 mg/L), and high (1000 mg/L) test concentrations.</p>

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		<p><i>EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.</i></p> <p><i>OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.</i></p>
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	HPLC at 0 and 96 hours; LOQ was 26.25 mg/L	
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:	21-21.5°C Continuous 8000 lux, fluorescent lighting	<p>The light intensity of 8,000 lux was greater than recommended (4,000-5,000 lux).</p> <hr/> <p><i>EPA temperature: <u>Skeletonema</u>: 20°C, Others: 24-25°C; EPA photoperiod: <u>S. costatum</u> 14 hr light/ 10 hr dark, Others: Continuous; EPA light: <u>Anabaena</u>: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)</i></p> <p><i>OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.</i></p>
<u>Reference chemical (if used)</u> name: concentrations:	Potassium dichromate 0.6, 1.0, 1.4, 1.8, and 2.2 mg/L	<p>The EC₅₀ was 0.7 (95% C.I.=0.7-0.8) mg/L for potassium dichromate.</p>
Other parameters, if any	N/A	

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2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks
		Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell density and area under the growth curve (biomass).	<i>EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.</i>
Measurement technique for cell density and other end points	Microscope with "Neubauer chamber" at 24 and 48 hours, and Spectrophotometer at 72 and 96 hours.	<i>EPA recommends the measurement technique of cell counts or chlorophyll a</i> <i>OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).</i>
Observation intervals	At 24, 48, 72, and 96 hours.	<i>EPA and OECD: every 24 hours.</i>
Other observations, if any	The pH was measured in each treatment group and control at 0 and 96 hours. The temperature range during testing was reported.	
Indicate whether there was an exponential growth in the control	Yes, the dilution water control cell densities at test termination were 242x greater than the dilution water control cell densities at test initiation.	<i>EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.</i> <i>OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.</i>
Were raw data included?	Yes, replicate data were provided.	

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II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By 96-hours, cell density percent inhibition was -2, 8, 4, 13, and 15% at the nominal 62.5, 125, 250, 500, and 1000 mg/L treatment levels, respectively, compared to the control. The area under the growth curve (biomass) percent inhibition was -6.5, 6.8, -0.9, 12.0, and 16.2% at the nominal 62.5, 125, 250, 500, and 1000 mg/L treatment levels, respectively, compared to the control. Biomass was determined by the study author to be significantly reduced compared to the control at the 1000 mg/L treatment level. The 96-hour NOAEC and LOAEC were 500 and 1000 mg/L.

Table 3: Effect of Chlormequat Chloride on algal growth (*Scenedesmus subspicatus*)

Treatment nominal and (measured) ^a concentrations (mg/L)	Initial cell density	Cell density at			
		24 hours	72 hours	96 hours	
				cell count	% inhibition ^b
Negative control	10,000	25,000	614,000	2,415,000	--
62.5 (47.9)	10,000	43,000	642,000	2,471,000	-2
125	10,000	33,000	578,000	2,217,000	8
250 (233)	10,000	33,000	628,000	2,326,000	4
500	10,000	29,000	538,000	2,110,000	13
1000 (899)	10,000	29,000	496,000	2,045,000	15

^a The test concentrations that were measured are presented in parentheses.

^b Negative percent inhibition indicates promoted growth.

Table 4: Effect of Chlormequat Chloride on algal growth (*Scenedesmus subspicatus*)

Treatment nominal and (measured) ^a concentrations (mg/L)	Initial cell density	Mean Area Under the Growth Curve	
		96 hours	% inhibition ^b
Negative control	10,000	4549	--
62.5 (47.9)	10,000	4846	-6.5
125	10,000	4240	6.8
250 (233)	10,000	4591	-0.9
500	10,000	4003	12.0
1000 (899)	10,000	3811	16.2*

^a The test concentrations that were measured are presented in parentheses.

^b Negative percent inhibition indicates promoted growth.

*Statistically significant percent reduction compared to the control (Dunnnett's test, $p \leq 0.05$).

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Table 5: Statistical endpoint values.

Statistical Endpoint	Cell density ^a	Growth rate	Biomass ^a
NOAEC or EC ₀₅ (mg/L)	Not determined	Not determined	500
EC ₅₀ (mg/L)	Not determined	Not determined	>1000
EC ₅₀ (mg/L) (95% C.I.)	Not calculable	Not determined	Not calculable
Other (IC ₂₅ /EC ₂₅)	Not determined	Not determined	Not determined
Reference chemical, potassium dichromate NOAEC EC ₅₀	Not determined	Not determined	Not reported 0.7 (0.7-0.8) mg/L

^a Based on nominal concentrations.

B. REPORTED STATISTICS:

The Dunnett-test was used to compare treatment groups to the control. The NOAEC was determined using these statistical results. The area under the growth curve formula incorporated cell density and time as variables. The EC₅₀ was estimated because the percent inhibitions were <50% for all endpoints. The statistical calculations were performed using the nominal concentrations.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density and biomass data were analyzed using the Chi-square and Shapiro-Wilks tests for normality and the Hartley and Bartlett's tests for homogeneity of variances. Data satisfied the assumptions of ANOVA, so the NOAEC values were determined using ANOVA (cell density), followed by William's test (biomass). These analyses were conducted using TOXSTAT statistical software. The EC_x values were determined using non-linear regression via Nuthatch statistical software. Where available, mean-measured concentrations were used to determine these estimates; nominal concentrations were used for levels that weren't measured.

Cell density:

EC₀₅: 140 mg/L 95% C.I.: 10-1800 mg/L
 EC₅₀: >898.9 mg/L 95% C.I.: Not calculable
 NOAEC: 898.9 mg/L
 Probit Slope: 0.834±0.477

Area under the growth curve (biomass):

EC₀₅: 210 mg/L 95% C.I.: 33-1300 mg/L
 EC₅₀: >898.9 mg/L 95% C.I.: Not calculable
 NOAEC: 232.6 mg/L
 Probit Slope: 1.18±0.641

Endpoint(s) Affected: Cell density and biomass

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D. STUDY DEFICIENCIES:

Treatment concentrations were not analytically verified for all levels tested. However, levels that were tested approximated nominal concentrations (74.1-94.0 % nominal) and showed little variability within and between sampling periods.

E. REVIEWER'S COMMENTS:

The reviewer's NOAEC determination for biomass was lower than the study author's; this difference is attributed to the different statistical methods used. The reviewer's analysis additionally provided EC₀₅ values and slopes for cell density and biomass endpoints. The reviewer also used mean measured concentrations, where available, while the study author based estimates on nominal concentrations. The reviewer's results are reported in the Executive Summary and Conclusions sections.

The purity of the active ingredient was not provided as a percentage, so the results could only be expressed as mg product/L.

The experiments started on April 24, 1989 and were completed on April 28, 1989.

F. CONCLUSIONS:

This toxicity study is scientifically sound and satisfies the guideline requirement for aquatic nonvascular plant toxicity study with the freshwater green algae species, *Scenedesmus subspicatus*. Neither endpoint was sensitive to treatment (i.e., exhibited inhibition in excess of 50%); the EC₅₀ was >898.9 mg/L. The NOAEC values for cell density and biomass were 898.9 and 232.6 mg/L. This study is classified as ACCEPTABLE.

Cell density:

EC ₀₅ :	140 mg/L	95% C.I.: 10-1800 mg/L
EC ₅₀ :	>898.9 mg/L	95% C.I.: Not calculable
NOAEC:	898.9 mg/L	
Probit Slope:	0.834±0.477	

Area under the growth curve (biomass):

EC ₀₅ :	210 mg/L	95% C.I.: 33-1300 mg/L
EC ₅₀ :	>898.9 mg/L	95% C.I.: Not calculable
NOAEC:	232.6 mg/L	
Probit Slope:	1.18±0.641	

Endpoint(s) Affected: Cell density and biomass

III. REFERENCES:

Miller, R.G.: Simultaneous Statistical Inference, Springer Verlag, New York (1981).

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density

File: 5222c

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	4299.324	859.865	2.992
Within (Error)	12	3448.547	287.379	
Total	17	7747.871		

Critical F value = 3.11 (0.05,5,12)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All groups equal

cell density

File: 5222c

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	241.500	241.500		
2	47.89	247.133	247.133	-0.407	
3	125	221.700	221.700	1.430	
4	232.6	232.633	232.633	0.641	
5	500	211.033	211.033	2.201	
6	898.9	204.533	204.533	2.671	*

Dunnett table value = 2.50 (1 Tailed Value, $P=0.05$, $df=12,5$)

cell density

File: 5222c

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	3			
2	47.89	3	34.604	14.3	-5.633
3	125	3	34.604	14.3	19.800
4	232.6	3	34.604	14.3	8.867
5	500	3	34.604	14.3	30.467
6	898.9	3	34.604	14.3	36.967

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cell density

File: 5222c

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	241.500	241.500	244.317
2	47.89	3	247.133	247.133	244.317
3	125	3	221.700	221.700	227.167
4	232.6	3	232.633	232.633	227.167
5	500	3	211.033	211.033	211.033
6	898.9	3	204.533	204.533	204.533

cell density

File: 5222c

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	244.317				
47.89	244.317	0.203		1.78	k= 1, v=12
125	227.167	1.036		1.87	k= 2, v=12
232.6	227.167	1.036		1.90	k= 3, v=12
500	211.033	2.201	*	1.92	k= 4, v=12
898.9	204.533	2.671	*	1.93	k= 5, v=12

s = 16.952

Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	1.4E+02	10.	1.8E+03	0.53	0.076
EC10	3.7E+02	80.	1.7E+03	0.31	0.21
EC25	2.0E+03	5.1E+02	7.8E+03	0.28	0.26
EC50	1.3E+04	4.6E+02	3.6E+05	0.68	0.036

Slope = 0.834 Std.Err. = 0.477

Goodness of fit: p = 0.50 based on DF= 3.0 12.

5222C : cell density

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Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	242.	244.	-2.36	100.	0.00
47.9	3.00	247.	239.	8.50	97.9	2.14
125.	3.00	222.	232.	-10.8	95.3	4.67
233.	3.00	233.	226.	6.62	92.7	7.32
500.	3.00	211.	215.	-3.58	88.0	12.0
899.	3.00	205.	203.	1.59	83.2	16.8

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

auc (biomass)

File: 5222b

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2296161.611	459232.322	5.572
Within (Error)	12	989057.333	82421.444	
Total	17	3285218.944		

Critical F value = 3.11 (0.05,5,12)

Since F > Critical F REJECT Ho:All groups equal

auc (biomass)

File: 5222b

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	4549.000	4549.000		
2	47.89	4845.333	4845.333	-1.264	
3	125	4240.333	4240.333	1.317	
4	232.6	4591.000	4591.000	-0.179	
5	500	4003.000	4003.000	2.329	
6	898.9	3811.000	3811.000	3.148	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

Data Evaluation Report on the Acute Toxicity of STABILAN (a.i. Chlormequat chloride) to *Scenedesmus subspicatus*

PMRA Submission Number {.....}

EPA MRID Number 467152-22

auc (biomass)

File: 5222b

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2			Ho:Control<Treatment		
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	3			
2	47.89	3	586.023	12.9	-296.333
3	125	3	586.023	12.9	308.667
4	232.6	3	586.023	12.9	-42.000
5	500	3	586.023	12.9	546.000
6	898.9	3	586.023	12.9	738.000

auc (biomass)

File: 5222b

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)			TABLE 1 OF 2		
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	4549.000	4549.000	4697.167
2	47.89	3	4845.333	4845.333	4697.167
3	125	3	4240.333	4240.333	4415.667
4	232.6	3	4591.000	4591.000	4415.667
5	500	3	4003.000	4003.000	4003.000
6	898.9	3	3811.000	3811.000	3811.000

auc (biomass)

File: 5222b

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)			TABLE 2 OF 2		
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	4697.167				
47.89	4697.167	0.632		1.78	k= 1, v=12
125	4415.667	0.569		1.87	k= 2, v=12
232.6	4415.667	0.569		1.90	k= 3, v=12
500	4003.000	2.329	*	1.92	k= 4, v=12
898.9	3811.000	3.148	*	1.93	k= 5, v=12

s = 287.091

Note: df used for table values are approximate when v > 20.

**Data Evaluation Report on the Acute Toxicity of STABILAN (a.i. Chlormequat chloride) to
*Scenedesmus subspicatus***

PMRA Submission Number {.....}

EPA MRID Number 467152-22

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	2.1E+02	33.	1.3E+03	0.37	0.16
EC10	4.2E+02	1.4E+02	1.3E+03	0.23	0.33
EC25	1.4E+03	6.1E+02	3.0E+03	0.16	0.45
EC50	5.1E+03	6.3E+02	4.1E+04	0.42	0.12

Slope = 1.18 Std.Err. = 0.641

Goodness of fit: p = 0.15 based on DF= 3.0 12.

5222B : auc (biomass)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	4.55e+03	4.65e+03	-98.4	100.	0.00
47.9	3.00	4.85e+03	4.61e+03	236.	99.2	0.824
125.	3.00	4.24e+03	4.52e+03	-275.	97.2	2.84
233.	3.00	4.59e+03	4.38e+03	206.	94.3	5.65
500.	3.00	4.00e+03	4.10e+03	-101.	88.3	11.7
899.	3.00	3.81e+03	3.78e+03	32.4	81.3	18.7

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.